

EFFECTS OF SO₂ POLLUTION ON THE SOIL MICROFLORA OF SPRUCE FOREST STANDSAleš LETTL⁺

The immissions influence microbial communities of the forest soil which are of special importance for maintaining the nutrient cycling. North-western Bohemia has become one of the most heavily polluted areas in Europe as far as sulfur immissions are concerned. Microbiological research in this country was therefore concentrated on the spruce forests in the Krušné Hory Mountains and in the Slavkovský Les Mountains /5/. A fine model area was found in South Moravia in the neighbourhood of the Fosfa factory near Poštorná, where a localized but intense immission load was recorded /13/.

Changes in the microflora of forest soils

One of the most striking observations was that of a decreased concentrations of bacterial colonization of the upper-most soil horizons. This is supported by data from South Moravia, where the counts of aerobic bacteria in a pine forest depended on the distance from the immission source /13/. This is in agreement with the results obtained in the spruce forests in the Slavkovský les Mts. Five areas were arranged according to increasing sulfate content in the fermentation horizon /this value is used as an auxiliary indicator of pollution/. Here, too, it is clear that the average bacterial concentrations in the F horizon decrease with immission intensity at elevations of 800 m as well as 500 m above sea level /5/.

On the other hand, the counts of micromycetes were increased in the fermentation horizon of the affected areas both in Moravia and in Bohemia /Tab. I/.

The SO₂ pollution appears to affect the species representation in the microbial communities, but precise data are lacking. *Pseudomonas* are the most bacterial cultures isolated from the fermentation horizon of spruce soils according to their production of a typical pigment. In addition, some other microorganisms were found that produced yellow, cream, ochre or red colonies but no attempt was made to identify them. The soil of the affected areas contains a markedly incre-

⁺ Forestry and Game Management Research Institute,
255 01 Zbraslav, Strnady 167, Czechoslovakia

ased proportion of such pigmented cultures, mainly at the expense of pseudomonads /9/. This phenomenon is much better expressed in the bacterial colonization of the spruce phylloplane. Even a light immission load completely eliminated not only pseudomonas, but also thiobacilli and asymbiotic nitrogen fixers. These bacteria were replaced by these yellow and red pigmented microorganisms /Tab.II/. Cultures resistant to toxic concentrations of sulfite at acid pH were found among them /6/.

The same is valid for the fungal communities: with increasing counts of microfungi a species reduction was observed, together with an increased incidence of the genera *Rhizopus* and *Mucor* /5, 13/.

The selection pressure of the pollution thus brings about species changes in the communities, suppressing the sensitive species and favouring the resistant microorganisms.

Soil biochemical activities

In the carbon cycle, the immissions markedly decrease the soil respiration of the F horizon. Results obtained in the spruce forests of the Slavkovský les Mts. exhibit a drop in the mean respiratory activity by 20 to 40%, depending on the elevation of the locality. A slower decomposition of cellulose was observed and a lower content of readily hydrolyzable saccharides was indicated. It thus appears that the decomposition of carbon compounds in forest soil is inhibited already at the level of high-molecular compounds /5/.

The nitrogen cycle is affected in a similar fashion. Ammonification of organic nitrogen is the activity that is most sensitive to immissions: in the affected spruce stands of the Slavkovský les Mts. it was depressed to the depth of mineral horizon A by 20 to 70% as compared with intact areas /Tab. I/.

Decreasing ammonification goes hand in hand with the amount of ammonium ions in the soil which may be related to a poorer nutrition and a decreased annual growth. However, in heavily affected soils an accumulation of the ammonium ion may be observed, without improving the microbial counts. This fact is now recognized as an inhibition of biological immobilization of nitrogen in organic matter /5/.

Nitrification was found under experimental conditions to be sensitive to immissions but in the acid soils of spruce forests nitrification is generally sporadic and its intensity is very low. Hence, it is difficult to demonstrate any effect of pollution in the field /5/. However, in vitro practically all cultures

of heterotrophic bacteria isolated from the fermentation horizon of spruce soils can oxidize ammonium ions to nitrates. Owing to the pollution the occurrence of this activity in bacterial communities is somewhat reduced. Oxidation of nitrites occurs at an approximately equal frequency. As to the amount of nitrates produced in vitro heterotrophic isolates from control /not polluted/ spruce soils are more efficient than isolates from affected areas. The difference is very distinct at elevations of about 800 m but relatively insignificant at 500 m above sea level. The results were obtained after four transfers of strains on standard media where a direct effect of immissions was excluded. This indicates that the bacterial populations underwent a long-lasting damage /9/.

The SO_2 pollution has a pronounced effect on the sulfur cycle, in this case a positive one. Sulfur arrives in the soil through precipitation principally as sulfate, through a direct sorption of sulfur dioxide by the soil, and in the shed needles of spruce trees containing both organic and inorganic sulfur, particularly in the polluted areas. The affected soils contain substantially more sulfates than the control. Sulfate sulfur is partially immobilized in the soil and bound through microbial activity into organic compounds. The humic and fulvic acids from polluted soils may contain twice the normal amount of sulfur /4/.

This organic, reduced sulfur in soil is accepted by microorganisms as a nutrient and hence microbial groups that can utilize sulfur are favoured. This means that higher concentrations of thiobacilli can be found together with a constant representation of *T. thioparus* and namely *T. thiooxidans* /10/. The latter species is generally found only in soils very rich in sulfur and, because of its stringent requirement it is considered as rare.

It was shown that the ability to oxidize elemental sulfur to sulfates is induced under these conditions even in heterotrophic organisms. In bacterial communities from polluted soils the incidence of sulfur oxidizers may increase to twice the control /Tab. III/. In some types of tree stands this ability was present in all the isolated bacterial cultures but no microfungi with this activity could be found /8/. The opposite situation may be observed in other ecosystems: bacterial sulfur oxidizers may be rare but a high incidence of oxidizing microfungi is typical /15/. These data suggest that under conditions of high sulfur input both thiobacilli and heterotrophic bacteria and microfungi can be ecologically important in the sulfur cycle.

These facts are reflected in soil activities. The affected soils contained higher concentrations of thiosulfate sulfurtransferase /rhodanese/ /15/ which

splits thiosulfate to sulfite and thiocyanate. It was observed that this enzyme, previously believed to occur only in thiobacilli and thiosulfate-oxidizing photolithotrophs is generally distributed in heterotrophic bacteria of spruce humus /7/. Moreover, its occurrence is not restricted to pseudomonads /8/ where it has been established earlier. In affected spruce soils the ability to oxidize elemental sulfur to sulfate is markedly increased while the oxidation of thiosulfate is slightly inhibited /10/. As the various reactions proceeded at different rates, some accumulation of sulfur oxidation intermediates in soil cannot be excluded. This was actually found in the affected areas where the soil contained high concentrations of not only sulfates but also of tetrathionate and thiosulfate and of elemental sulfur on the surface of plants /14/.

The mechanism of pollution effect

Under conditions where the major component of pollution is SO_2 /further oxidized to sulfuric acid/, the H^+ ion and the SO_4^{2-} anion /followed by other sulfur compounds/ are considered as the main harmful agents. The primary symptom of contamination is then an acidification of the environment, together with its enrichment in sulfur.

SO_2 exhalations are generally associated with the occurrence of acid rain, which is considered as a very serious phenomenon. However, a number of data indicate that its importance may be overestimated. The experimental approaches are usually oriented on the acute effect during a treatment of soil with artificial acid rain. In all instances was it necessary to reduce pH drastically to 2.0 to obtain significant results. Irrigation with water of pH 3.0 was practically ineffective. This holds for the counts of heterotrophic bacteria, decomposition of organic matter and activities of soil enzymes. There in Slavkovský les Mts., the pH of precipitations ranged from 3.6 to 5.8. Passage of rain water through the canopy of the spruce stand led to a decrease of the lower limit to pH 3.2 /11/.

Another instance of disagreement can be observed: the average pH values of the unaffected and polluted forest soils differ very insignificantly. The following of this values for 9 years merely showed that pH of organic horizons /fermentation and humus/ oscillated about a steady value. Only the mineral A horizon was acidified more rapidly in the affected areas. At the same time, a decreasing trend in the bacterial colonization could be observed only in the F horizon. In the H horizon the effect of pollution was not evident. In the mineral A horizon /the only one that is progressively acidified/ the affected areas exhibited in fact increased bacterial counts. On the other hand, soil respiration and

ammonification were inhibited to the depth of the A horizon /Table I/, hence again irrespective of the mean soil pH /5/.

Bacterial communities of spruce soils became adapted to the lower pH. When evaluating the growth parameters of bacteria from soils with different pH values the conclusion can be drawn that the optimum pH for growth decreases. Hence, in acid forest soils neutrophilic bacteria are suppressed in favour of acidotolerant or acidophilic species. Not even this change must necessarily cause an impoverishing of the bacterial community as to its biochemical activities. Two hundreds acidophilic and neutrophilic isolates were tested for 8 decomposing or mineralizing activities. Acidophilic cultures, isolated and tested at pH 4, possessed practically all the activities of neutrophilic cultures, isolated and tested at pH 6 and 8 /Table IV/. Moreover, the acidophilic bacteria were more powerful nitrifiers than the neutrophilic ones /9/.

Acidification is certainly not a negligible factor. However it follows from these data, that, in the existing range or gradual acidification of soil in a hitherto known degree, soil pH values probably do not represent the decisive factors in the pollution effects on soil bacterial communities. The possibility cannot be excluded that the acidification of a soil milieu is more substantially affected by the spruce stand itself rather than by the composition of rain water.

If acidification alone cannot account for all the negative effects of pollution some authors mention a little hesitantly the accumulation of toxic derivatives of SO_2 as the key factor. It was shown above that in affected soils with a high sulfur content its cycle is intensified. Thus, a possibility is not excluded that the soil microflora could be damaged by all the intermediates of the sulfur cycle.

However, sulfates may be considered as nontoxic. Highly toxic sulfides cannot be the case in the well aerated upper horizons of forest soils. The minute production and the high volatility of carbon disulfide do not make the attainment of toxic levels probable. On the dermatology elemental sulfur has an antibacterial effect but the toxicity was due to sulfides formed in situ. With thiosulfate and tetrathionate a certain phytotoxicity was established but it could be demonstrated only under aseptic conditions. It disappeared if these substances were applied to soil directly.

Only sulfites exhibited a number of toxic effects. Their toxicity was found to be pH-dependent and increased in the acid region /1/. This pH-dependence was observed even during the effect of sulfites on the total community of soil bacte-

ria. A lower concentrations of sulfites /especially at higher pH/ have a distinctly stimulating effect on bacterial growth. In spite of this, irrigation of the acid spruce soil with a sulfite solution in amounts corresponding to the total immission load of sulfur in affected areas decreased the bacterial counts but did not markedly affect the biochemical activities of soil, of which especially the ammonification was sensitive to immissions. Sulfites were thus unable to simulate the effect of immissions /9/. Moreover, these compounds are extremely unstable in the soil milieu: their oxidation to sulfates and their binding to organic matter proceeds very rapidly, the phenomenon being mainly abiotic /2/. It is also possible that increased numbers of sulfite-oxidizing thiobacilli and the sulfite oxidase activity demonstrated in various heterotrophic bacteria may play a partial role here. The instability of sulfite in the soil environment decrease the probability of attaining its toxic concentration. Analyses of a lot of soils proved its concentration only in ppm, differences between pure and polluted samples being negligible /16/. However, it may be of importance in this context that polluted spruce soils contained a higher percentage of heterotrophic bacteria capable of growth at toxic concentrations of sulfite in the medium /Table V/. This sulfite-resistance was found not only in soil bacteria but also in those colonizing the spruce phylloplane in polluted areas /6/. The existence of sulfite-resistant bacteria may have an anamnestic significance and it forces us to admit, hesitantly to be sure, that sulfite may have any effect in the affected soils.

Of the inorganic compounds coming into play here SO_2 itself should be mentioned. Its high toxicity has been well demonstrated in a lot of papers. Fumigation of soil with SO_2 imitates best the effect of immissions in all respects examined. Sulfur dioxide will penetrate several centimeters into the soil where it is avidly sorbed /12/. In exposed localities the forest soil receives as much as 70% of its sulfur input in this way /3/. The extent of sorption increases with soil humidity, an aqueous solution of sulfur dioxide being clearly more toxic than a solution of sulfite.

In the whole sulfur cycle there is no other substance as effective and characteristic as SO_2 to account for the effect of immissions. It thus appears reasonable to ascribe the majority of pollution effects to sulfur dioxide, with a possible participation of sulfites.

Further development of soil microbial communities

The ecosystem of spruce stands has been studied predominantly for the econo-

mic importance of spruce as well as for its sensitivity to pollution. The existing knowledge of the effect of immissions on soil microbial communities is thus biased. Extrapolation of the negative effects observed in the spruce forest soils gives rise to pessimistic prognoses. A high /even though local/ intoxication is assumed for soil, leading to a total destruction of the microbial life. However, in fact the whole ecosystem changes. The dead spruce stands are replaced with resistant vegetation, mainly with grassy stands of mountain ash and birch, which has obvious consequences in the state of the soil microflora as a component of the ecosystem.

Mainly the grass cover gives rise to a pronounced rhizosphere effect which is best reflected in the concentrations of heterotrophic bacteria. These concentrations in a mountain ash stand lie at values that are higher by one order of magnitude than those in the grass-free soil of a spruce stand. In contrast, micromycetes decrease in numbers. Soil respiration is only slightly increased but the nitrogen cycle is intensified and nitrification appears during the growing season.

A comparison of the incidence of decomposing and mineralizing activities was made in cultures of heterotrophic bacteria isolated from the fermentation horizon of three types of stands: spruce, withered spruce with grass cover and grassy mountain ash stand. The occurrence of activities increased from the spruce stand to the mountain ash stand /Table VI/. Bacterial strains from the grassy mountain ash stand were thus equipped with a substantially wider spectrum of biochemical activities. If in a spruce soil most of the bacterial isolates possessed only two or three types of biochemical activities, in the mountain ash soil most cultures possessed ten or eleven types of activities of the total of twelve tested. The incidence of enzyme producers was increased, the production of the three enzymes tested was more intense in vitro. The powerfully developed microbial community was thus richly equipped with activities necessary for normal soil function /9/.

Conclusions

Several effects of SO_2 pollution on the soil microflora of spruce stands have been described, most of them negative. Most of them were actually observed as statistically insignificant trends. The microflora can adapt, at least to a certain extent, to the affected environment. Now it is recognized that changes in species composition, increased, acidophility, rise of resistance and induction

of biochemical activities in the microbial communities can play a role in this context. The potentialities of the microbial community are greater than those of the spruce stand. The trees are destroyed before the soil microflora is critically damaged. Death of spruce forests and the subsequent grass covering then induces a dramatic improvement of the microbial situation.

Under the present immission load it is unlikely that soil would become sterile and biological processes would cease. Clearly, the principal reason for the death of spruce trees in affected areas is not a major alteration of soil microflora.

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Table I. Average numbers of microorganisms /in thousands per g oven dry soil/ and soil biochemical activities /in mg of CO₂ and NH₄⁺-N per kg soil in 24 h and 14 d, respectively/ in the /F/ fermentation, /H/ humus and A horizons of spruce forest soils /West Bohemia, Slavkovský les Mts. /5/.

Elevation above sea level /m/	800		500		
Character of areas	unaffected	slightly polluted	unaffected	strongly polluted	strongly polluted
<u>Horizon F</u>					
SO ₄ ²⁻ content /μg/g/	203	249	237	309	383
pH /H ₂ O/	3.70	3.56	3.82	3.86	3.78
pH /KCl/	2.97	2.82	3.19	3.35	3.11
aerobic bacteria	888	730	3 683	2 537	1 450
ammonifying bact.	1 198	1 068	4 255	4 554	1 501
micromycetes	488	736	780	1 110	694
respiration	2 223	1 729	2 360	1 385	1 392
ammonification	115	89.8	147	79.1	49.2
<u>Horizon A</u>					
pH /H ₂ O/	3.50	3.45	3.52	3.55	3.55
pH /KCl/	3.00	2.87	2.91	3.07	2.72
aerobic bacteria	187	234	65.9	108	194
ammonifying bact.	394	323	133	241	338
micromycetes	194	184	51.9	52.2	170
respiration	176	210	132	70	104
ammonification	6.4	17.4	12.5	6.4	5.0

Table II.

Pigmentation in heterotrophic bacterial communities from soils and spruce phylloplane in pure and polluted areas /soils sampled on January 12, 1983; apical shoots sampled on August 30, 1982

Elevation above sea level /m/	Character of areas	Total number of isolates	Per cent of cultures	
			yellow, ochre, red pigmented	yellow-green pigmented /pseudomonads/
<u>Spruce forest soils</u>				
800	unaffected	26	19.2	80.8
	polluted	25	52.0	48.8
500	unaffected	20	20.0	80.0
	polluted	25	44.0	56.0
<u>Spruce phylloplane</u>				
800	unaffected	56	0	100
	slightly polluted	20	100	0
500	polluted	17	100	0
	polluted	20	100	0

Table III. Occurrence of sulfur oxidizers in soils of spruce forest stands on the /THA/ Thornton agar and /MPA/ meat-peptone agar.

Elevation above sea level	Character of areas	Average numbers ^a of thiobacilli	Per cent of heterotrophic bacteria ^b oxidizing S ⁰ to SO ₄ ²⁻ on the medium	
			THA, pH 4.0	MPA, pH 6.0 - 8.0
800	pure unaffected	109 ± 63.4 /4/	24.0	52
	slightly polluted	10.6 ± 5.6 /4/	-	-
	polluted	4 000 ^c	59.2	88
500	unaffected	63.6 ± 82.5 /5/	29.6	45
	polluted	398 ± 280 /5/	68.2	80
	polluted	283 ± 233 /5/	-	-

^a $\bar{X} \pm S_{\bar{X}} /n/$; in thousands per g oven dry soil

^b Analysis of isolates from samples taken on January 12, 1983

^c Date from one sample taken on June 3, 1982

Table IV. Biochemical activities of heterotrophic bacteria, isolated on Thornton /THA/ and meat-peptone agar /MPA/ from F horizon of spruce forest soils /sampling on January 12, 1983/.

Elevation above sea level	800				500			
Character of areas	unaffected		polluted		unaffected		polluted	
Medium	THA	MPA	THA	MPA	THA	MPA	THA	MPA
pH	4.0	6.0	4.0	6.0	4.0	8.0	4.0	8.0
Total number of isolates	27	26	27	25	27	20	23	25
Per cent of isolates with the activity								
Cellulolysis	88.9	84.6	81.5	92	3.7	15	0	36
Amylolysis	11.1	69.2	7.4	36	18.5	25	0	28
Gelatinolysis	0	3.8	0	0	3.7	30	30.4	40
Ammonification of asparagine	92.6	92.3	88.9	96	100	100	95.6	84
Oxidation of NH_4^+ to NO_3^-	100	80.8	66.7	64	100	95	91.3	68
Reduction of S^0 to S^{2-}	0	38.5	8.7	4	0	50	0	48
Oxidation of S^0 to SO_4^{2-}	24	52.2	59.2	88	29.6	45	68.2	80
Oxidation of $\text{S}_2\text{O}_3^{2-}$ to SO_4^{2-}	11.1	0	29.6	0	33.3	35	0	16

Table V. Occurrence of bacteria growing on Thornton agar /pH 5.0/ with sulfite in spruce forest soils

Elevation above sea level /m/	Horizon	Character of areas	Per cent of colonies ^a on THA with sulfite concentration /mmol/l/		
			1.0	3.0	10.0
800	F	unaffected	74.6	2.86 ^b	0.38 ^c
		polluted	71.4	12.2 ^b	1.12 ^c
	H	unaffected	41.7	8.66	3.15
		polluted	39.1	10.7	6.90
500	F	unaffected	54.0 ^d	18.4	0.15 ^c
		polluted	99.6 ^c	38.0	1.38 ^c
	H	unaffected	69.0 ^b	6.34 ^c	1.41 ^b
		polluted	97.3 ^b	43.2 ^c	10.8 ^b

^a Thornton agar without sulfite = 100%

Differing at ^b P = 0.1, ^c P = 0.05, ^d P = 0.01

Table VI. Occurrence of biochemical activities in bacterial cultures, isolated on MPA /pH 7.0/
from soils of three types of forest stands /soil sampling on October 5, 1982/

Quantity	Type of forest stand		
	spruce	withered spruce	mountain ash
Total number of isolates	24	22	28
Per cent of isolates with the activity			
Cellulolysis	16.7	68.2	78.6
amylolysis	37.5	59.1	96.4
gelatinolysis	8.3	45.4	96.4
ammonification	62.5	86.4	100
of asparagine			
oxidation of	0	13.6	64.3
NH_4^+ to NO_3^-			
oxidation of	58.3	81.8	71.4
NO_2^- to NO_3^-			
reduction of S^0 to S^{2-}	29.2	54.5	92.9
oxidation of S^0 to SO_4^{2-}	91.7	81.8	100
oxidation of	25.0	54.5	100
$\text{S}_2\text{O}_3^{2-}$ to SO_4^{2-}			
Production of			
thiosulfate sulfurtransferase /rhodanese/	8.3	45.4	92.9
production of	29.2	59.1	64.3
thiosulfate oxidase			
production of	8.3	13.6	25.0
sulfite oxidase			